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Divergent roles for ferric ions in the biological activity of amidated and non-amidated gastrins.

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Amidated forms of the peptide hormone gastrin act via the cholecystokinin-2 receptor to stimulate gastric acid secretion, whereas non-amidated forms stimulate colonic mucosal proliferation via a novel, as yet uncharacterised, receptor. Nuclear magnetic resonance (NMR) and fluorescence spectroscopic studies have revealed that glycine-extended gastrin17 bound two ferric ions, and that ferric ion binding was essential for biological activity. We have therefore investigated the role of ferric ions in the biological activity of amidated gastrin17. As with glycine-extended gastrin17, fluorescence quenching experiments indicated that Glu7 Ala and Glu8,9 Ala mutants of amidated gastrin17 each bound only one ferric ion. The affinity of the mutant peptides for the cholecystokinin-2 receptor on transfected COS-7 cells or on Tlymphoblastoid Jurkat cells, and their potency in stimulation of proliferation in Jurkat cells and inositol phosphate production in transfected COS-7 cells, were similar to the values obtained for amidated gastrin17. In addition, the iron chelator desferrioxamine did not significantly inhibit either binding of amidated gastrin17 to the cholecystokinin-2 receptor, or stimulation of inositol phosphate production by amidated gastrin17 in transfected COS-7 cells. We conclude that, in contrast to glycine-extended gastrin17, binding of ferric ions is not essential for the biological activity of amidated gastrin17. Our results support the concept of distinct modes of action for amidated and non-amidated gastrins, and raise the possibility of developing selective antagonists of the actions of non-amidated and amidated gastrins.

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by Satsangi *et al* in the initial analysis.[13] As concluded by Lesage and colleagues[2] further studies are needed to determine if chromosome 12 contains a major disease gene or if the putative gene in this region has only a small influence on the risk of developing IBD.

A third possibility is that we are looking at a population specific difference. Although it appears unlikely that the population in the UK is substantially different from that in France or Belgium, only a repeat study in the UK could clarify this issue. Within our own linkage study[9] much of the power for the weak linkage seen on chromosome 12 resulted from the sib pairs recruited from the UK which would support this theory (unpublished). A consortial analysis, which drew about 50 sib pairs from each sample collected world wide,[22] confirmed linkage to chromosome 12 although at a much lower significance level than that reported initially.

The work of Lesage and colleagues[2] further indicates that linkage to chromosome 12 is not as powerful as initially thought. Identification of the first disease genes for IBD is still eagerly awaited. Although most likely in the distant future, only the definition of the causative mutations will allow a precise construction of a disease model and more exact predictions of the number and influence of additional genetic alterations.

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Gastrin, growth, and colon neoplasia

The ability of the gastric hormone gastrin to stimulate gut epithelial cell proliferation has been appreciated since the late 1960s.[1] However, aside from the special case of gastric carcinoid tumours arising from enterochromaffin-like cells, the contribution of gastrin to gastrointestinal neoplasia has been uncertain. Several developments now suggest a role for gastrin in both gastric and colorectal cancer. In the case of gastric cancer, recent evidence indicates a synergy between gastrin and *Helicobacter* infection in accelerating progression to atrophy and cancer.[2] Different issues are involved in colorectal cancer. The important emerging concepts here are that (a) the gastrin gene is expressed in colorectal cancer cells, but (b) the main products of gene expression in these cells are not ligands for the gastrin-cholesystokinin receptor B (CCKB), although (c) they do act as colon growth factors. The paper by Smith and Watson[3] now shows that gastrin mRNA, detected by reverse transcription-polymerase chain reaction, is expressed in early stage polyps, that the main forms of gastrin detected by immunohistochemistry are biosynthetic precursor peptides, and that there is also parallel expression of the gastrin-CCKB receptor (see page 820).

It used to be thought that the only biologically active peptides generated from the gastrin gene were COOH terminally amidated peptides such as G34 and G17, and that carboxy terminal amidation was essential for the biological activity of members of the gastrin family. The amidated gastrins are generated from a precursor, progastrin, via biosynthetic intermediates, the Gly-gastrins (fig 1). Neither progastrin nor Gly-gastrins have a carboxy terminal amide group and therefore they do not have high affinity for the gastrin-CCKB receptor, which is the receptor mediating acid secretory responses to gastrin. It seems, however, that these peptides exhibit other biological properties, notably stimulation of colon cell growth. This effect has been found in both transgenic mice over expressing progastrin and Gly-gastrin, and in colon cancer cell lines.[4–6] The precise receptors mediating the trophic actions of progastrin and Gly-gastrin remain uncertain. It is not clear if there is one (the putative gastrin-CCKC receptor) or several. It is conceivable that a modified version of the gastrin-CCKB receptor, perhaps generated by alternative mRNA splicing, mediates the effects of progastrin and Gly-gastrin; at present this idea is largely speculative. It is unlikely, however, that the progastrin and Gly-gastrin found by Smith and Watson in early stage polyps act at authentic gastrin-CCKB receptors.

The pattern of relatively high abundance of progastrin and Gly-gastrin compared with amidated gastrin that occurs in colonic neoplasms differs markedly from that in normal pyloric antral G cells where the amidated gastrins predominate. There are several plausible explanations for this. There may, for example, be differences in the expression of enzymes required for each conversion step in

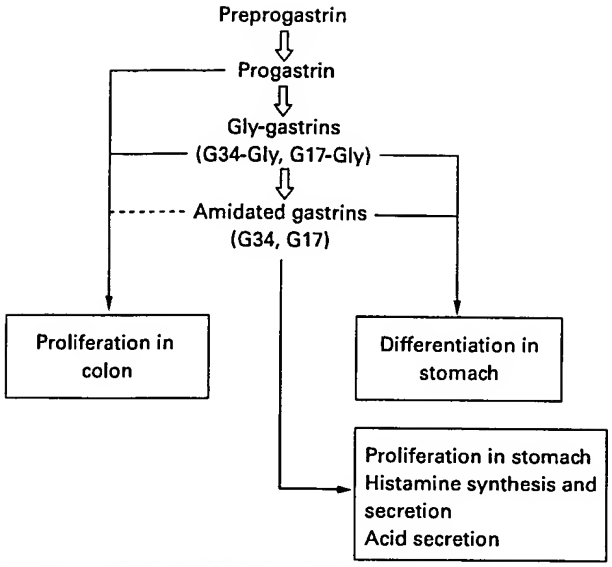

Figure 1 Schematic representation of the pathway of gastrin biosynthesis and the biological properties of the products. Open arrows show the conversion steps by which preprogastrin is converted to amidated gastrins. This sequence of events is completed in G cells but in colorectal cancer cells the main products are progastrin and Gly-gastrin. Progastrin and Gly-gastrins stimulate colon proliferation. Amidated gastrins play a minor role in control of colon proliferation but stimulate acid secretion and gastric proliferation via the gastrin-CCKB receptor (at which progastrin and Gly-gastrin have low affinity).

gastrin biosynthesis (fig 1). In normal G cells, the enzymes responsible for production of carboxy terminal amidated gastrins mostly act in vesicles of the so called regulated pathway of exocytosis[7]; but these vesicles are scarce or absent in polyp and adenoma cells. As a consequence, these cells are poorly equipped both to store hormonal peptides and to complete the full sequence of events giving rise to carboxy amidated gastrins. Instead, peptides mostly corresponding to progastrin and Gly-gastrin are likely to pass directly from the Golgi complex to the cell surface by what is known as the constitutive route of secretion.

What regulates gastrin gene expression in colon polyps and adenomas? Studies by Nakata *et al* have shown that oncogenic Ras stimulates the mitogen activated protein kinase pathway and so increases gastrin gene expression.[8] Recently, Koh *et al* demonstrated that the β-catenin/T cell factor 4 pathway also stimulates gastrin gene expression.[9] Acquisition of mutations that activate the latter pathway is likely to be an early event in the progression to colorectal cancer and could account for the increased gastrin gene expression found by Smith and Watson. Whether or not similar mechanisms account for upregulation of the gastrin-CCKB receptor is not known.

Recently, Singh *et al* reported that mice with elevated plasma progastrin exhibit increased aberrant crypt foci, adenomas, and adenocarcinomas after treatment with azoxymethane.[10 11] Aberrant crypt foci are considered to be a marker for progression to colon cancer. Singh *et al* suggest progastrin is a cocarcinogen, that is, on its own it is not carcinogenic but it increases the pool of transformed cells and so exacerbates the oncogenic progression. Interestingly, mice with elevated plasma concentrations of amidated gastrin did not exhibit increased aberrant crypt foci, adenomas, or carcinomas in response to azoxymethane. The picture emerges then that mutations acquired early in the progression to colorectal cancer lead to increased local production of progastrin and Gly-gastrin that then act as auto- or paracrine growth factors expanding the number of transformed cells. This analysis leaves open the role of the gastrin-CCKB receptor, and further work will certainly be needed on this point. Whether or not these findings can be developed into novel therapeutic strategies will depend on characterisation of the receptor mechanisms mediating the effects of progastrin or Gly-gastrin on colon epithelial proliferation.

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Alcohol and retinoid metabolism

The complex interactions between the metabolism of retinoids and ethanol have been reported for a long time. Clinically, chronic ethanol consumption leads to vitamin A deficiency but also to enhanced toxicity of vitamin A and beta-carotene when supplemented. Changes in retinol metabolism due to alcohol may have a pathophysiological impact in both alcoholic liver disease and alcohol associated cancer as retinoic acid, the most active form of vitamin A, is an important regulator of normal epithelial cell growth, function, and differentiation. Under normal conditions, ingested retinol is metabolised to retinaldehyde via cytosolic alcohol dehydrogenase (ADH), microsomal retinol dehydrogenase (three types), and several types of cytosolic retinol dehydrogenases, and retinaldehyde is further oxidised to retinoic acid via aldehyde dehydrogenase (ALDH). Retinoic acid binds to retinoic acid receptors (RAR), initiating intracellular signal transduction leading to a cascade of events and finally to a decrease in cell regeneration. The main molecular action of retinoic acid involves either transactivation through direct binding to retinoic acid response elements (RARE) in target gene promoters, thereby transcriptionally activating a series of genes with distinct antiproliferative activity, or transrepression of activator protein (AP-1) and regulation of apoptosis. It is not surprising that this complex interaction between ethanol and metabolism of retinoids occurs as